

CLAIMS

What is claimed is:

5           1.    A method of introducing a specific mutation into a selected DNA molecule for mutagenesis, wherein said DNA molecule is a double-stranded circular DNA molecule, said method comprising the steps of:

                  annealing a first mutagenic primer and a  
10           second mutagenic primer to said DNA molecule, wherein said first mutagenic primer comprises a region that is complementary to the second mutagenic primer and wherein said first and second mutagenic primers,

15           synthesizing by means of a linear cyclic amplification reaction a first mutagenized DNA strand comprising said first mutagenic primer, and a second mutagenized DNA strand comprising said second mutagenic primer, wherein the first  
20           mutagenized DNA strand and the second mutagenized DNA may form a double-stranded mutagenized circular DNA intermediate, and

                  digesting said DNA molecule for mutagenesis, wherein said digestion is mediated by a selection  
25           enzyme.

                  2.    The method according to Claim 1, wherein said selection enzyme digests methylated DNA strands and said selected DNA molecule for mutagenesis is  
30           methylated.

                  3.    The method according to Claim 2, wherein said selected DNA molecule for mutagenesis is methylated in vivo.

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4. The method according to Claim 2, wherein said selected DNA molecule for mutagenesis is methylated *in vitro*.

5 5. The method according to Claim 1, wherein the selection enzyme is a restriction endonuclease.

6. The method according to Claim 2, wherein the selection enzyme is DpnI.

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7. The method according to Claim 1, wherein the linear cyclic amplification reaction is catalyzed by Pfu DNA polymerase.

15 8. The method according to Claim 1, wherein the first and second mutagenic primers are 5' phosphorylated.

9. The method according to Claim 1, wherein the  
20 linear cyclic amplification reaction is repeated for less than 20 cycles.

10. The method according to claim 1, wherein the first and second mutagenic primers are completely  
25 complementary to each other.

11. The method according to claim 1, said method further comprising the steps,

annealing said first mutagenized DNA  
30 strand and the second mutagenized DNA strand so as to form a double-stranded mutagenized circular DNA intermediate, and  
transforming a host cell with said double-stranded mutagenized circular DNA  
35 intermediate.

12. A kit for introducing a specific mutation into a selected DNA molecule for mutagenesis, said kit comprising: a DNA polymerase, a selection enzyme, control first and second mutagenic primers, and  
5 control templates.

13. A kit according to Claim 13, said kit further comprising competent cells.

10 14. A kit according to Claim 14, said kit further comprising concentrated reaction buffers.

15 15. A kit according to Claim 13, wherein said DNA polymerase is Pfu DNA polymerase.

16. A kit according to Claim 13, wherein said selection enzyme is a restriction endonuclease.

17. A kit according to Claim 17, wherein said  
20 restriction endonuclease is Dpn I.

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